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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/920,653	08/03/2001	Masaharu Noda	31671-173164	6043

26694 7590 09/25/2003

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EXAMINER	
TON, THAIAN N	

ART UNIT	PAPER NUMBER
1632	18

DATE MAILED: 09/25/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/920,653	NODA ET AL.	
	Examiner	Art Unit	
	Thái-An N. Ton	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *26 June 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-34* is/are pending in the application.

4a) Of the above claim(s) *5-23 and 25-34* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4,24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *08 November 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *6,9,16* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Applicants' Response, with regard to the Notice to Comply with Sequence Listing Requirement, filed 6/26/03, Paper No. 17, is proper and has been entered.

Claims 1-34 are pending. Claims 1-4 and 24 are under current examination.

Election/Restrictions

Applicant's election of Group I [Claims 1-4 and 24] in Paper No. 14 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claims 5-23 and 25-34 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected groups, there being no allowable generic or linking claim. Election was made **without** traverse in Paper No. 14.

Specification

The disclosure is objected to because of the following informalities: The specification refers to claim numbers, which is improper. See pp. 4-7. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-4 and 24 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a mouse whose genome comprises a homozygous disruption of the exon 1 of the endogenous Na_v2 gene, wherein the disruption results in a phenotype of normal salt intake under water sufficient conditions, when compared to wild-type mice, but shows an increase of hypertonic saline under water and salt-depleted conditions, when compared to a wild-type mouse and methods of screening using the knockout mouse, the specification does not reasonably provide enablement for a null mutant non-human animal characterized in showing salt intake behavior similar to that of wild-type animals under water-sufficient conditions and showing much more intakes of hypertonic saline compared with wild-type animals under water and salt-depleted conditions, and methods of using the same. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The claimed invention is directed to a null mutant non-human animal characterized in showing salt intake behavior similar to that of wild-type animals under water sufficient conditions and showing much more intakes of hypertonic saline compared with wild-type animals under water- and salt-depleted conditions and methods of screening a material utilizing the null mutant non-human animal.

The specification teaches that the Na_v2 knockout mice can be made by screening a mouse genomic DNA library using a cDNA that codes for rat NaG, and then constructing a targeting vector which can then be introduced into ES cells by electroporation. After homologous recombination, the chimeric mice are intercrossed to produce heterozygous mice, which are then crossed to generate knockout Na_v2 mice. See p. 9, 2nd ¶. Particularly, the specification teaches a targeting vector containing fragments encoding exons 1, 2 and 3 of Na_v2 mouse gene. The targeting vector introduced into mouse ES cells by electroporation and neomycin-resistant ES clones were selected and homologous recombination was verified by Southern blot. The positive ES cell clones were injected into mouse embryos which were cultured to the blastocyst stage and transplanted into a recipient mouse. The resulting chimeric mice were mated with wild-type mice to produce heterozygotes, and the heterozygotes were intercrosses to produce Na_v2 knockout mice. See pp. 20-23. The physiological role of the mouse Na_v2 gene was examined. Postnatal animals were perfused and fixed brains were analyzed. See pp. 25-29.

The behavior of the Na_v2 knockout mice was examined by analysis of water and salt intake of the mice. Particularly, the mutant mice were backcrossed with wild-type mice to verify that the F1 and F4 behavior was identical. The mice first underwent a two-bottle preference test, wherein the mice (-/-, +/- and +/+) were presented with both distilled water and a tasting solution. The total intake for each

animal was measured to calculate a preference ratio. It was found that the knockout mice showed normal preferences to various tasting solutions under conditions satiated with salt and water. See pp. 29-30. The taste responses were verified by electrophysiological analysis, where it was found that the knockout responses were similar to those found in wild-type mice. The same mice were tested under water-depleted conditions to examine the preference to hypertonic saline before and after a 24-hour dehydration. The mice were trained to drink water from two bottles for one week prior to the testing. On the day of dehydration, the mice were presented with water and 0.3 M NaCl a 10 h, and then measured for fluid intake at 16 hours. At 10 hours on the next day, the bottles were removed and dry food was presented through the period of water deprivation. After the 24-hour dehydration period, the two bottles were returned and fluid intakes were measured at 16 hours. It was found that the knockout Na_v2 mice had an abnormal ingestion of the hypertonic saline under water-depleted (dehydrated) condition and that in contrast to wild-type and heterozygous mutant mice, the null mutants showed no change in preference ratios to hypertonic saline after dehydration. Blood was recovered from the animals before or after dehydration and concentrations of plasma electrolytes measured. It was found that the electrolyte concentrations in the serum before and after the dehydration were normal in both wild-type and homozygous mutant mice. The specification teaches that this suggests that the Na_v2 knockout mice excreted excessive amounts of sodium into urine, and thus the

renal function of these mice is normal. The specification teaches that a motivated salt appetite was induced by an intraperitoneal injection of a diuiretic drug. Firstly, a group of mice were injected intraperitoneally with saline and the bottle of 0.3 NaCl was withdrawn and sodium-depleted food was supplied in place of a normal diet. A second injection of normal saline was given. On the following day, water and 0.3 M of NaCl were present and intakes of NaCl and water measured. A similar protocol was followed including the injection of the diuiretic drug, furosemide. These results were compared with normal sodium-containing food. It was found that there was a significant difference between the wild-type and knockout Na_v2 mice. Under the acute salt appetite condition induced by the furosemide injection, the knockout Na_v2 mice showed an approximately 2-fold increase in the ingestion of 0.3 M NaCl when compared to the wild-type and heterozygous mutant mice, and this abnormal ingestion stopped when sodium-containing food was provided. See pp. 32-34.

The standard under 112, 1st ¶ entails the determination of what the claims recite, and what the claims mean as a whole. In addition, in analysis of the enabled scope of the claims, the teachings of the specification are taken into account because the claims are to be given their broadest reasonable interpretation that is consistent with the specification. As such, in light of the instant specification, the claimed invention is properly interpreted with regard to the disclosed phenotype of the exemplified Na_v2 (-/-) mice. Such an interpretation is consistent with the teachings

of specification because, with regard to the enablement requirement, one of skill in the art must be provided with both how to make and how to use the claimed invention. Note that the specification fails to teach any other disruptions in any other exon of the Na_v2 gene that result in the claimed phenotype. As such, the enabled scope of the claimed invention, in light of the teachings of the specification, is found to be a mouse whose genome comprises a homozygous disruption of exon 1 of the endogenous Na_v2 gene, wherein the disruption results in a phenotype of normal salt intake under water sufficient conditions, when compared to wild-type mice, but shows an increase of hypertonic saline under water and salt-depleted conditions, when compared to a wild-type mouse.

The specification teaches methods that require ES cells, however, the state of the art is such that ES cell technology is generally limited to the mouse system at present, and that only "putative" ES cells exist for other species (see Moreadith *et al.*, **J. Mol. Med.**, 1997, p. 214, S*ummary*). Note that "putative" ES cells lack a demonstration of the cell to give rise to germline tissue or the whole animal, a demonstration which is an art-recognized property of ES cells. Moreadith *et al.* supports this observation as they discuss the historical perspective of mouse ES cells as follows:

> "The stage was set-one could grow normal, diploid ES cells in culture for multiple passages without loss of the ability to contribute to normal development. Furthermore, the cells contributed to the development of gametes at a high frequency (germline competence) and the haploid genomes of

> these cells were transmitted to the next generation. Thus, the introduction of mutations in these cells offered the possibility of producing mice with a predetermined genotype."

Such a demonstration has not been provided by the specification or the prior or post-filing art with regard to the generation of any species of animal ES cells, other than the mouse, which can give rise to the germline tissue of a developing animal. In addition, prior to the time of filing, Mullins *et al.* (**Journal of Clinical Investigation**, 1996) report that "[A]lthough to date chimeric animals have been generated from several species including the pig, in no species other than the mouse has germline transmission of an ES cell been successfully demonstrated." (page 1558, column 2, first paragraph). As the claims are drawn to non-human animals produced by methods which require the manipulation of embryonic stem cells, and particularly since the subject matter of the specification and the claimed invention encompasses the use of such cells for the generation of a knockout animal, the state of the art supports that only *mouse ES cells* were available for use for production of the claimed null mutant mice.

This is further supported by Pera *et al.* [**Journal of Cell Science** 113: 5-10 (2000)] who present the generic criteria for pluripotent ES or EG cells [see p. 6, 2nd column] and state that, "Thus far, only mouse EG or ES cells meet these generic criteria. Primate ES cells meet the first three of the four criteria, but not the last. Numerous other candidate mammalian ES cells have been described over the years

in domestic and laboratory species, but only in the mouse have all criteria been met rigorously." [See p. 6, 2nd column, last paragraph].

Furthermore, some of the claims recite that the null mutant non-human animal is a rodent. See claim 3. It is noted that the breadth of the term "rodent" encompasses various that include beavers and squirrels. See Encyclopedia Britannica, http://www.search.eb.com/dictionary. As stated previously, mammalian ES cells have not been described from species other than mouse; as such, the claimed null mutant rodents are not enabled.

The enabled scope of the claimed invention is also based on the unpredictable state of the transgenic knockout art in that disruption of a different exon of the same gene may not result in the anticipated phenotype. See Moreadith et al. (Journal of Molecular Medicine, 1997) who support phenotypic unpredictability in knockout mice. In particular, Moreadith et al. discuss that gene targeting at a particular loci is unpredictable with respect to the resulting phenotype since often the generation of knockout mice, in many instances, changes the prevailing notions regarding the functions of the encoded proteins. For example, Moreadith et al. report that gene targeting at the endothelin loci led to the creation of mice with Hirschsprung's disease instead of the anticipated phenotype (abnormal control of blood pressure). See page 208, column 2, 2nd paragraph.

Sanford *et al.* [**Meth in Mol. Bio**, 158:217-225, 2001] state that, "Once a knockout allele generated by gene targeting has been introduced into the germline

of a mouse, the primary concern is to efficiently screen the animal for mutant phenotypes. This is not necessarily a trivial exercise given the high frequency of unexpected or lack of phenotypes." [See p. 217, 1st paragraph]. Sanford *et al.* discuss various factors which have been shown to affect the phenotype, such as the genetic backgrounds of mice, which have been shown to influence the phenotype in the resulting knockout mouse [see p. 218 and Table 1]. Other factors which affect phenotype in knockout mice include a variation in penetrance, expressivity and modifier genes which are dependent upon genetic background.

Accordingly, in view of the quantity of experimentation necessary for the production and use of null mutant non-human animals, for the breadth claimed, the lack or direction or guidance, as well as absence of working examples, provided by the specification for the production and use of null-mutant non-human animals comprising a disruption in the Na_v2 knockout mammals, other than the exemplified Na_v2 knockout mouse, wherein the disruption is in exon 1 of the Na_v2 gene, the unpredictable and undeveloped state of the art for the production of knockout non-human animals, particularly with respect to the phenotypic effect, and the breadth of the claims encompasses null-mutant non-human animals, it would have required undue experimentation for one of skill in the art to make and/or use the claimed non-human animals and methods of using the same.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-4 and 24 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The term "much more" in claim 1 is a relative term which renders the claim indefinite. The term "much more" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. It is unclear how much intake of hypertonic saline would be considered "much more". Appropriate correction is required. Claims 2-4 and 24 depend from claim 1.

Claim 2, as written, is unclear. The claim recites that the in the null mutant animal, the function of the Na_v2 gene is deficient on "its chromosome". It is unclear what "its chromosome" refers to – the animal's chromosome (in which case, it would be expected that the animal would have more than one chromosome), or the chromosome on which the Na_v2 gene is found. Appropriate correction is required. Claims 2-3 and 4 depend from claim 4.

Claim 24, as written, is unclear. The claim is drawn to a method of screening a material that promotes or suppresses the function or the expression of a protein acting as a sensor of extracellular sodium ion level characterized in using the non-human animal according to claim 1, and a subject material. However, no clear and

defined steps are recited in the independent claims. While minute details are not required in method claims, at least the basic steps must be recited in a positive, active fashion. See Ex Parte Erlich, 3 USPQ2d, p. 1011 (Bd. Pat. App. Int. 1986).

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Thái-An N. Ton whose telephone number is (703) 305-1019. The examiner can normally be reached on Monday through Friday from 8:00 to 5:00 (Eastern Standard Time), with alternating Fridays off. Should the examiner be unavailable, inquiries should be directed to Deborah Reynolds, Supervisory Primary Examiner of Art Unit 1632, at (703) 305-4051. Any administrative or procedural questions should be directed to William Phillips, Patent Analyst, at (703) 305-3482. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703)-872-9306.

TNT

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